

PCT

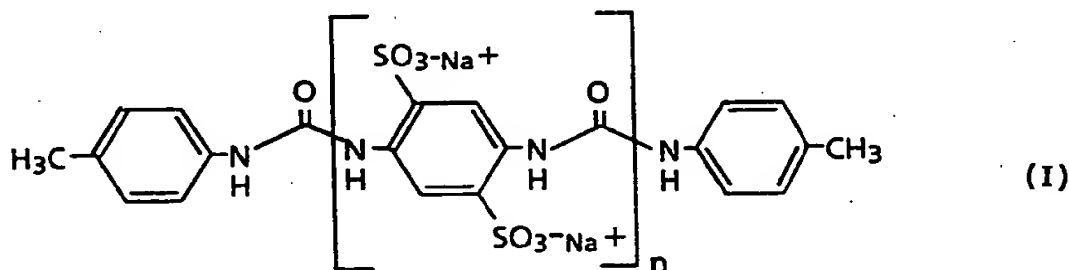
WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification <sup>5</sup> : <b>A61K 31/795</b></p>	<p><b>A1</b></p>	<p>(11) International Publication Number: <b>WO 94/07507</b> (43) International Publication Date: <b>14 April 1994 (14.04.94)</b></p>
<p>(21) International Application Number: <b>PCT/US93/08168</b> (22) International Filing Date: <b>30 August 1993 (30.08.93)</b> (30) Priority data: <b>07/952,393</b>                      <b>28 September 1992 (28.09.92)</b> <b>US</b> (71) Applicant: <b>MERRELL DOW PHARMACEUTICALS INC. [US/US]; 2110 East Galbraith Road, P.O. Box 156300, Cincinnati, OH 45215-6300 (US).</b> (72) Inventor: <b>STAUDERMAN, Kenneth, A. ; 1343 Meier Avenue, Cincinnati, OH 45208 (US).</b> (74) Agent: <b>SAYLES, Michael, J.; Marion Merrell Dow Inc., 2110 East Galbraith Road, P.O. Box 156300, Cincinnati, OH 45215-6300 (US).</b></p>		<p>(81) Designated States: <b>AU, CA, FI, HU, JP, KR, NO, NZ, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).</b>  <b>Published</b> <i>With international search report.</i> <i>With amended claims.</i></p>

(54) Title: METHOD FOR ANTAGONIZING INOSITOL 1,4,5-TRIPHOSPHATE



(57) Abstract

Oligomers of formula (I) have been demonstrated to be effective antagonists of inositol 1,4,5-triphosphate (IP<sub>3</sub>) by competitively vying with IP<sub>3</sub> for binding sites. By competitively inhibiting the activity of IP<sub>3</sub>, the oligomers of this invention can modulate the release of intracellular calcium and elicit the resultant physiological effects.

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FR	France	MR	Mauritania
AU	Australia	GA	Gabon	MW	Malawi
BB	Barbados	GB	United Kingdom	NE	Niger
BE	Belgium	GN	Guinea	NL	Netherlands
BF	Burkina Faso	GR	Greece	NO	Norway
BG	Bulgaria	HU	Hungary	NZ	New Zealand
BJ	Benin	IE	Ireland	PL	Poland
BR	Brazil	IT	Italy	PT	Portugal
BY	Belarus	JP	Japan	RO	Romania
CA	Canada	KP	Democratic People's Republic of Korea	RU	Russian Federation
CF	Central African Republic	KR	Republic of Korea	SD	Sudan
CG	Congo	KZ	Kazakhstan	SE	Sweden
CH	Switzerland	LJ	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovak Republic
CM	Cameroon	LU	Luxembourg	SN	Senegal
CN	China	LV	Latvia	TD	Chad
CS	Czechoslovakia	MC	Monaco	TG	Togo
CZ	Czech Republic	MG	Madagascar	UA	Ukraine
DE	Germany	ML	Mali	US	United States of America
DK	Denmark	MN	Mongolia	UZ	Uzbekistan
ES	Spain			VN	Viet Nam
FI	Finland				

-1-

5

METHOD FOR ANTAGONIZING INOSITOL 1,4,5-TRISPHOSPHATE

10

BACKGROUND OF THE INVENTION

This application relates to a series of diamino benzenedisulfonic acid oligomers that have demonstrated an affinity for the receptor sites of inositol 1,4,5-  
15 triphosphate (IP<sub>3</sub>) and are, therefore useful in diminishing the bioactivity of IP<sub>3</sub>, especially with regard to its effect on the release of intracellular calcium ions.

DESCRIPTION OF THE PRIOR ART

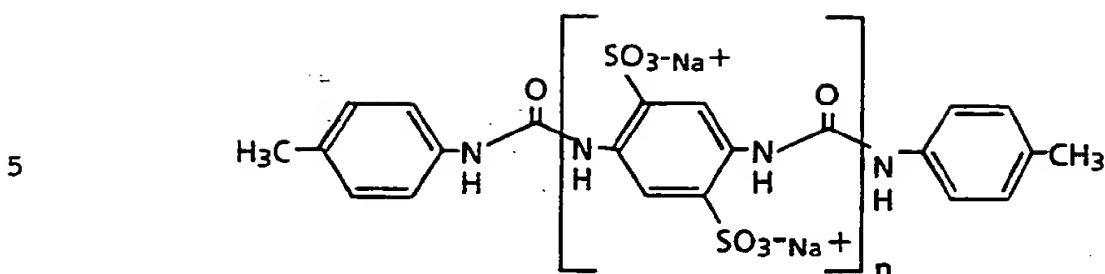
20 The diamino benzenedisulfonic acid oligomers demonstrating utility as IP<sub>3</sub> antagonists according to this invention are described in detail in the European Patent Application published January 22, 1992 under Publication No. 0467185 A2. In that publication, the oligomers of the  
25 present invention were described as having utility in the diagnosis and/or treatment of AIDS and AIDS related complex.

SUMMARY OF THE INVENTION

30 The invention herein disclosed relates to a method of inhibiting the activity of inositol 1,4,5-triphosphate (IP<sub>3</sub>) by occupying the receptor sites specific to IP<sub>3</sub> with a compound of the formula:

35

-2-



10 wherein n is a whole number selected from the range of 5 to 20 inclusive and the pharmaceutically acceptable salts thereof.

#### DETAILED DESCRIPTION OF THE INVENTION

15 Inositol 1,4,5-triphosphate (IP<sub>3</sub>) is a naturally occurring and active component of animal physiology. It is formed intracellularly upon the activation of cell-surface receptors linked to the enzyme phospholipase C. Once generated in sufficient quantities, IP<sub>3</sub> acts to stimulate the release of calcium ions from storage organelles within the cell. In this role IP<sub>3</sub> is characterized as a "second messenger". Depending upon the type of cell, the calcium released by IP<sub>3</sub> functions to stimulate a variety of physiologic processes such as smooth muscle contraction, histamine secretion and the hyperpolarization of nerve cells. Any compound or agent that can promote or interfere with the function of IP<sub>3</sub>, will promote or interfere with the generation of calcium ions and thereby elicit predictable pharmacological effects.

30 The process by which IP<sub>3</sub> releases calcium ions begins with the binding of IP<sub>3</sub> to a specific receptor protein located on an intracellular calcium storage compartment located typically on the endoplasmic reticulum. This receptor protein has been cloned and has been shown to form a calcium "channel" with unique structural properties when bound to IP<sub>3</sub>. Therefore, when IP<sub>3</sub> binds with its receptor, a calcium channel is opened causing the release of calcium stored in the cell's endoplasmic reticulum. In turn, the

-3-

released calcium will elicit the appropriate cellular response.

Heretofore, the only verified potent antagonist of the IP<sub>3</sub> receptor was heparin, a complex glycosaminoglycan. The diamino benzenedisulfonic acid oligomers of this invention also appear to antagonize the effects of IP<sub>3</sub> by competing for the receptor site. In most cases, these compounds are more effective than heparin and demonstrate fewer secondary effects. In addition to providing utility as laboratory "tools" in evaluating the therapeutic potential of other IP<sub>3</sub> receptor antagonists, the oligomers of this invention would also be administered to modulate IP<sub>3</sub>-induced calcium release and have a salutary effect on any number of disorders that are caused or exacerbated by an inordinately productive IP<sub>3</sub> second messenger pathway.

#### EXPERIMENTALS

##### Measurement of IP<sub>3</sub> Binding

Cerebella from male Sprague-Dawley rats (200 g) were homogenized in 30 volumes of ice-cold buffer A (50 mM Tris, 1 mM dithiothreitol, 1 mM EDTA, pH 7.7 with HCl) with a polytron (setting 9 for 10 seconds). The tissue is then washed twice by centrifugation (20,000 x g, 15 minutes; Sorvall 28-S., SS-34 rotor) and resuspended in 30 volumes of ice-cold buffer A.

For the binding assays, 1.5 ml eppendorf tubes containing 50 µl of test compound (made up as a 10x stock in water) or water, 50 µl [3H] IP<sub>3</sub> (17Ci/mmol; Dupont-NEN; usually made as a 25 nM (10x) stock solution in buffer), and 350 µl of buffer B (buffer A with pH adjusted to 8.4) on ice. Tubes for non-specific binding also contained 50 µl of non-radioactive IP<sub>3</sub> (100 µM stock (10x); final concentration 10 µM), with an appropriate reduction in the volume of buffer B. Reactions were initiated by the addition of 50 µl tissue to make the final volume 500 µl,

-4-

followed by vortex mixing. Samples were incubated on ice for 10 minutes and then were centrifuged (14,000 x g) in a microfuge (Eppendorf model 5415) for 5 minutes followed by aspiration of the supernatant fraction. The tissue pellets  
5 were solubilized overnight in 100  $\mu$ l of Protosol (Dupont-NEN). After solubilization, 73  $\mu$ l of glacial acetic acid were added to decrease chemiluminescence, and the mixture was transferred to scintillation vials. To these vials was added 7 ml of Ecoscint-A (National Diagnostics) and the  
10 radioactivity determined by liquid scintillation spectrophotometry.

Specific binding was defined as the difference between total binding (radioactivity in the absence of test  
15 compound and cold  $IP_3$ ) and non-specific binding (radioactivity in the absence of test compound but in the presence of cold  $IP_3$ ). This number was taken as 100% specific binding. Data points obtained with the test compounds were fit by a computer program (GraphPad-InPlot)  
20 to determine their inhibitory potency. The inhibitory potencies of the test compounds were expressed as the concentration of compound that produces 50% inhibition of specific binding (the  $IC_{50}$  value).

25 The binding data are presented in Table 1 and demonstrate that compounds within the scope of the present invention effectively compete for [ $^3H$ ]  $IP_3$  binding sites in rat cerebellar membranes. The compound identified as MDL 102,869 was the most potent competitor for binding with an  
30  $IC_{50}$  of 50 nM, whereas low molecular weight heparin (5100 MW) had an  $IC_{50}$  of 74 nM. MDL 102,869 is the compound according to the claimed invention wherein n = 15.

The potency for binding also seems to correlate with  
35 the ability to antagonize  $IP_3$ -induced calcium ion release. Thus, 1 and 3  $\mu$ M of MDL 102,869 inhibited calcium ion release by 42 and 100%, respectively (see Fig. 1), whereas

-5-

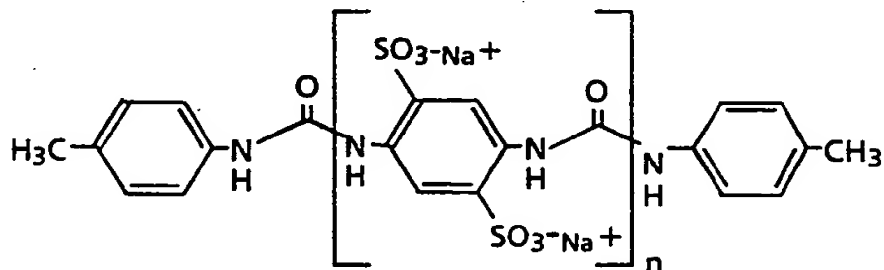
10  $\mu\text{M}$  of heparin inhibited release by 90%. MDL 101,828, which had an  $\text{IC}_{50}$  binding of 104 nM, inhibited  $\text{IP}_3$ -induced calcium ion release by 72% at 3 micro moles. MDL 101,828 is the compound according to the claimed invention wherein

5  $n = 9$ .

TABLE 1

Compound	Binding $\text{IC}_{50}$ (nM)	Inhibition of $\text{Ins}(1,4,5)\text{P}_3$ -Induced $\text{Ca}^{2+}$ -release
Heparin (low MW)	74	90% at 10 $\mu\text{M}$
MDL 101,828	104	42% at 3 $\mu\text{M}$
MDL 102,869	50	42% at 1 $\mu\text{M}$ 100% at 3 $\mu\text{M}$

15 The tracing of Fig. 1, dramatically illustrates the  $\text{IP}_3$  inhibition data set forth in the third column of Table 1.



The y-axis represents the concentration of free calcium ions in arbitrary units. The tracing shows that two successive additions of 0.1  $\mu\text{M}$  of  $\text{IP}_3$  stimulated similar amounts of calcium ion release from cerebellar microsomes.

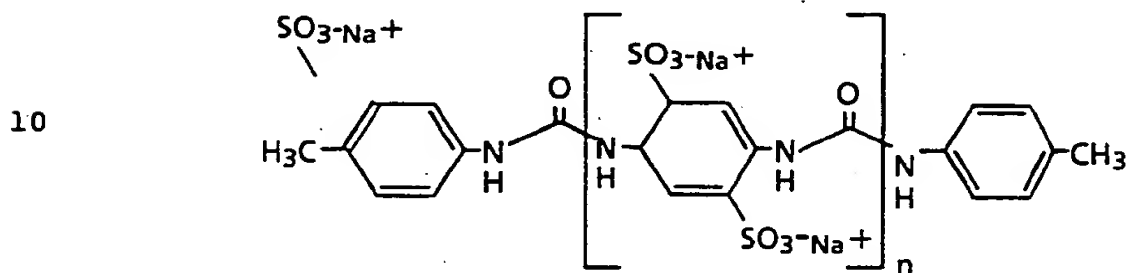
30 The addition of 1  $\mu\text{M}$  of MDL 102,869 stimulated a small increase of calcium ion for unknown reasons. In the presence of 102,869, however, calcium ion release stimulated by 0.1  $\mu\text{M}$  of  $\text{IP}_3$  was inhibited by 42%. This inhibition was overcome by the addition of 1  $\mu\text{M}$  of  $\text{IP}_3$ ,

35 consistent with competitive antagonism by MDL 102,869.

-6-

## WHAT IS CLAIMED IS:

1. A method of inhibiting the activity of inositol 1,4,5-trisphosphate by occupying a receptor site specific to inositol 1,4,5-trisphosphate with a compound of the formula:



wherein n is a whole number within the range of 5-20 and the pharmaceutically acceptable salts thereof.

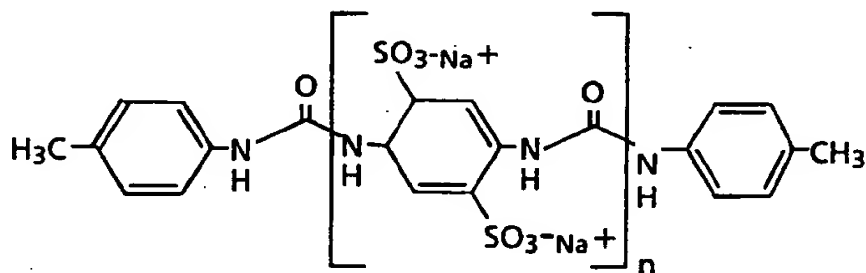
2. The oligomer of claim 1 wherein n is 9.
- 20
3. The oligomer of claim 1 wherein n is 15.
- 25
- 30
- 35



## AMENDED CLAIMS

[received by the International Bureau on 11 January 1994 (11.01.94);  
original claims 2-3 replaced by amended claims 2-3; other claims unchanged  
(1 page)]

1. A method of inhibiting the activity of inositol  
5 1,4,5-trisphosphate by occupying a receptor site specific  
to inositol 1,4,5-trisphosphate with a compound of the  
formula:



wherein n is a whole number within the range of 5-20  
and the pharmaceutically acceptable salts thereof.

- 20 2. The method of claim 1 wherein n is 9.
3. The method of claim 1 wherein n is 15.
- 25
- 30
- 35

## INTERNATIONAL SEARCH REPORT

Int. Application No  
PCT/US 93/08168

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 5 A61K31/795

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 5 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP,A,0 467 185 (THE DOW CHEMICAL COMPANY) 22 January 1992 cited in the application	1
X	see abstract; claims; example 3 ---	2,3
Y	THE BIOCHEMICAL JOURNAL vol. 267, no. 2, 1990 pages 297 - 302 F. O'ROURKE ET AL. 'THE INOSITOL 1,4,5-TRISPHOSPHATE RECEPTOR BINDING SITES OF PLATELET MEMBRANES' see the whole document --- -/--	1

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \*&\* document member of the same patent family

Date of the actual completion of the international search

14 December 1993

Date of mailing of the international search report

23.12.93

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax (+31-70) 340-3016

Authorized officer

Hoff, P

# INTERNATIONAL SEARCH REPORT

Inter. nal Application No  
PCT/US 93/08168

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>FEBS LETTERS vol. 252, no. 1,2 , 1989 pages 105 - 108 M.A. TONES ET AL. 'THE EFFECT OF HEPARIN ON THE INOSITOL 1,4,5-TRISPHOSPHATE RECEPTOR IN RAT LIVER MICROSOMES' see the whole document ----</p>	1
A	<p>THE JOURNAL OF BIOLOGICAL CHEMISTRY vol. 263, no. 23 , 1988 pages 11075 - 11079 T.K. GHOSH ET AL. 'COMPETITIVE, REVERSIBLE, AND POTENT ANTAGONISM OF INOSITOL 1,4,5-TRISPHOSPHATE-ACTIVATED CALCIUM RELEASE BY HEPARIN' see the whole document ----</p>	1
A	<p>GB,A,781 479 (CIBA LIMITED) 21 August 1957 see page 2, line 8 - line 30; claims 1,2,9-12,22; example 5 -----</p>	1-3

# INTERNATIONAL SEARCH REPORT

Int. application No.

PCT/US 93/08168

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Although claim 1 is directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

Inter. Application No

PCT/US 93/08168

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0467185	22-01-92	AU-B- 635850	01-04-93
		AU-A- 8024291	09-01-92
		AU-A- 8286791	04-02-92
		CA-A- 2046491	10-01-92
		CN-A- 1058959	26-02-92
		EP-A- 0538373	28-04-93
		JP-A- 4226521	17-08-92
		WO-A- 9200749	23-01-92
<hr/>			
GB-A-781479		NONE	
<hr/>			

THIS PAGE BLANK (uspro)